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Two-sample Mendelian randomization analysis reveals causal relationships between blood lipids and venous thromboembolism

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Author Contributions

YZ, WH and KZ performed the data analyses and wrote the manuscript. YZ, SHT, HW, PFW and WH generated figures for the manuscript. HZX, KZ, YG and TLY designed, coordinated, and supervised the project. YG and TLY revised the manuscript.

Competing interests

The authors declare no competing interests.

Data-sharing statement

The summary data for 139 lipid GWAS is derived from the concept validation of the cross-platform mGWAS (https://omicscience.org/apps/crossplatform/). The GWAS data for the remaining 98 lipids and related traits was obtained from the website (https://gwas.mrcieu.ac.uk/datasets/?gwas_id__icontains=met-c). The MVP lipid GWAS results are available in dbGAP. The dbGAP accession number for MVP overall is phs001672.v4.p1. The GWAS summary statistics of VTE, DVT of lower extremities and PE were accessed from the FinnGen study (https://finngen.gitbook.io/documentation/data-download).

Abstract

Venous Thromboembolism (VTE) is a complex disease that can be classified into two subtypes: Deep Vein Thrombosis (DVT) and Pulmonary Embolism (PE). Previous observational studies have shown associations between lipids and VTE, but causality remains unclear. Hence, by utilizing 241 lipid-related traits as exposures and data from the FinnGen consortium on VTE, DVT, and PE as outcomes, we conducted two-sample Mendelian randomization (MR) analysis to investigate causal relationships between lipids and VTE, DVT and PE. The MR results identified that fatty acid (FA) unsaturation traits (Ratio of bis-allylic bonds to double bonds in lipids, and Ratio of bis-allylic bonds to total fatty acids in lipids) were associated with VTE (OR [95% CI]: 1.21 [1.15-1.27]; 1.21 [1.13-1.30]), DVT (OR [95%CI]: 1.24 [1.16-1.33]; 1.26 [1.16-1.36]) and PE (OR [95%CI]: 1.18 [1.08-1.29]; 1.18 [1.09-1.27]). Phosphatidylcholines exhibit potential causal effects on VTE and PE. Phosphatidylcholine acyl-alkyl C40:4 (PC ae C40:4) was negatively associated with VTE (OR [95% CI]: 0.79 [0.73-0.86]), while phosphatidylcholine diacyl C42:6 (PC aa C42:6) and phosphatidylcholine acyl-alkyl C36:4 (PC ae C36:4) were positively associated with PE (OR [95%CI]: 1.44 [1.20-1.72]; 1.22 [1.10-1.35]). Additionally, we found that medium LDL had a protective effect on VTE. Our study indicates that higher FA unsaturation may increase the risk of VTE, DVT, and PE. Different types of phosphatidylcholine have either promotive or inhibitory effects on VTE and PE, contributing to a better understanding of the risk factors for VTE.

Keywords: Venous thromboembolism, Deep vein thrombosis, Pulmonary embolism, Lipids, Mendelian randomization, Phosphatidylcholine, Fatty acid unsaturation

Introduction

Venous thromboembolism (VTE) is a highly prevalent and complex chronic disease, which can manifest specifically as deep vein thrombosis (DVT) and pulmonary embolism (PE) based on the location of the blood clot.[1] DVT occurs when a blood clot forms in the deep veins of the leg or pelvis. Once part of the clot detaches and enters the pulmonary artery through the circulation, it may lead to PE. PE can be life-threatening due to oxygen deprivation and circulatory failure.[2] VTE is influenced by environmental and genetic factors, and various factors such as aging, major surgery, prolonged immobility, malignancies, and obesity may affect the risk of VTE.[3,4] Approximately 10 million people are affected by VTE annually, making it the third largest vascular disease after acute myocardial infarction and stroke, contributing significantly to the global disease burden.[1]

Lipids are a common and diverse class of compounds that play important biological functions in various aspects, including serving as structural components of cell membranes, energy storage sources, and participating in signaling pathways.[5] The different blood lipids and lipoproteins have pro-coagulant and anticoagulant functions, implying that blood lipids may be linked to venous thrombosis.[6] Previous observational studies[7-9] have reported that the fluctuation of lipids was associated with VTE. For example, a population-based case-control study found that elevated triglyceride (TG) was associated with an increased risk of venous thrombosis in postmenopausal women, while higher level of high-density lipoprotein cholesterol (HDL-C) was associated with a decreased risk.[7] Another study compared plasma lipid profiles between patients with post-VTE and those without VTE, and revealed phosphatidylcholine (PC) and TG were higher in most of the historical patients with VTE.[8] Jiang et al investigated 240 cases of venous thromboembolism (including 125 cases of pulmonary embolism) and 6963 controls. They found a significant association between C5 carnitine and VTE events, while confirming elevated levels of diacylglycerol in VTE and PE patients.[9] Although a large amount of evidence confirming the association between blood lipids and VTE, most studies are observational and subject to sample size limitations and confounding factors. The causal link between blood lipids and VTE remains unclear.

Two-sample Mendelian randomization (MR) utilizes genetic variants of the exposure as instruments to estimate the potential causal association between exposures and outcomes. MR provides a reliable method to assess the causal relationship between genetic risk factors and phenotypic outcomes from a genetic perspective, which ensures that the estimation is less likely to be influenced by environmental confounding.[10] Based on this robust method, the genome-wide association studies (GWAS) with large sample size have identified multiple genetic variations on lipids and lipid-related traits, which may provide a great deal of genetic instrumental variables for causality estimation.[11] Several MR studies have been conducted to evaluate the causal relationship between lipids and VTE.[10,12-14] Lin et al utilized bidirectional MR analysis to investigate the relationship between three classical lipids (low-density lipoprotein (LDL), high-density lipoprotein (HDL), and TG) and VTE, and found no significant causal association.[12] Another two-sample MR study exploring the causal relationship between five circulating lipids (apolipoprotein A1, apolipoprotein B, LDL, HDL, and TG) and DVT also yielded similar conclusions.[14] MR studies investigating the causality between fatty acids (FA) and VTE suggest that different types of FA have different inhibitory or protective effects on VTE.[10,13] However, most of MR studies focus on only a subset of lipids, and currently, causal relationship between lipids and the risk of VTE still need to be confirmed in larger samples.

In this study, we conducted two-sample MR analyses to investigate the causal effects between blood lipids and VTE, DVT and PE, respectively. The lipids and lipid-related traits including phosphatidylcholine, sphingomyelin, acylcarnitine, fatty acids, lipoproteins, and others. Our findings provide new insights into the relationship between endogenous lipid metabolism and VTE, and contribute to a better understanding of the risk factor for VTE from a genetic perspective.

Methods

Study design

We performed a two-sample MR study to explore the possible causal effects of 241 blood lipids and lipid-related phenotypes on VTE, DVT and PE, respectively. The outline of the study design is shown in **Figure 1.**

GWAS data sources

A total of 241 blood lipids and lipid-related phenotypes were used as exposures (***Online Supplementary Table S1***), with 139 lipids derived from proof-of-concept cross-platform GWAS study.[15] This study provided GWAS summary data for each metabolite with sample sizes ranging from 8,569 to 86,507 individuals. In addition, 98 lipids and lipid-related traits were derived from a study published in 2016.[16] The sample sizes for different metabolites in this study ranged from 13,000 to 19,000. Finally, the GWAS summary statistics of low-density lipoprotein cholesterol (LDL-C), HDL-C, total cholesterol (TC) and TG were obtained from the Million Veteran Program (MVP), which involved up to 215,551 European individuals. The "aa" and "ae" denote that fatty acids are bound to the glycerol backbone via ester or ether bonds. The x and y in "x:y" indicate the number of carbon atoms and double bonds in the FA chain of the lipids. The outcome data for VTE, DVT and PE were available from the FinnGen study, comprising 377,277 individuals (19,372 cases and 357,905 controls), 333,230 individuals (9,109 cases and 324,121 controls) and 376,351 individuals (9,243 cases and 367,108 controls), respectively. All data sets used in this study had been approved by a relevant ethical review board.

Data filtration and genetic instruments selection

We filtered all GWAS summary datasets according to the following steps: 1) removing SNPs located in the major histocompatibility complex (MHC) region; 2) removing SNPs with minor allele frequency (MAF) less than 0.01; 3) removing palindrome SNPs with alleles A/T or G/C and MAF close to 0.5. According to the three assumptions that MR genetic instrumental variables (IV) must satisfy, we selected SNPs that are genome-wide significance (P value threshold $< 5 \times 10^{-8}$), not in linkage equilibrium (r^2 threshold > 0.001, window size = 1000kb), and free of weak instrument bias (F-statistic > 10). The RadialMR package was used to remove outlier pleiotropic SNPs.[17] After IV selection, we harmonized the effect alleles and adjusted β values in the outcome data to make it consistent with the exposure data.[18]

Statistical analyses

Consistent with our previous studies,[19,20] we conducted two-sample MR analyses using five methods, including inverse-variance weighted (IVW), robust adjusted profile score (RAPS), MR-Egger, weighted median and weighted mode, with IVW as the primary method. All MR analyses were implemented by the TwoSampleMR R package.[21] The intercept of MR-Egger regression can be used to detect the

pleiotropy in MR estimates.[22] Cochran's Q statistic (Q) and Rucker's Q statistic (Q') are used to assess heterogeneity in IVW and MR-Egger estimates, respectively.[23] The difference between the two Q statistics (Q-Q') can be used to assess the horizontal pleiotropy of the MR estimates, while a *P* value of less than 0.05 for the Q statistics and Q-Q' indicates the presence of directional pleiotropy.[24] Leave-one-out (LOO) sensitivity analysis is conducted to detect the presence of potential dominant SNPs. We also applied the Bonferroni correction to adjust for multiple testing. Complete details of genetic instruments selection and MR analysis are available in the ***Online Supplementary Methods.***

Results

The MR estimates of blood lipids and lipid-related phenotypes on VTE

The complete results of MR analyses and pleiotropy assessment for 187 blood lipids and lipid-related traits on VTE are shown in ***Online Supplementary Table S2.*** We identified 20 blood lipids and related lipid-related traits that were causally associated with VTE according to the IVW method, of which 11 were phosphatidylcholines, 1 was lysophosphatidylcholine, 5 traits related to FA saturation, and 3 traits related to medium LDL (*P* value < 2.67×10^{-4}, ***Online Supplementary Table S2*).** The pleiotropy assessment showed that no significant evidence of pleiotropy was detected by the Cochran's Q test and the intercept of the MR-Egger method (*P* value > 0.05). However, there were 6 phosphatidylcholines (PC aa C36:4, PC ae C36:3, PC ae C36:2, PC ae C38:2, PC ae C42:3, PC aa C34:4) with Q-Q' differences that were extreme enough to suggest the presence of directional pleiotropy (*P* < 0.05**, *Online Supplementary Table S2***). Sensitivity analysis for remaining 14 blood lipids and lipid-related traits showed that 8 of them had major influential SNPs driving causal estimates, suggesting that the significant MR estimates for these blood lipids and lipid-related traits are not robust in terms of causal effects (***Online Supplementary Figure S1***).

After excluding 14 exposures that exhibit directional pleiotropy or sensitivity, we ultimately identified 1 phosphatidylcholine (PC ae C40:4) and 3 traits related to medium LDL (Total lipids in medium LDL, Concentration of medium LDL particles, Total cholesterol in medium LDL) had protective effects on VTE, two FA saturation-related traits (Ratio of bis-allylic bonds to double bonds in lipids, Ratio of bis-allylic bonds to total fatty acids in lipids) showed pathogenic effects (**Figure 2**). These results were also validated in MR-RAPS (5/6) and weighted median (3/6) methods at the threshold of *P* value < 2.67×10^{-4}, and partially validated in weighted mode (6/6) and MR-Egger (3/6) methods with the threshold of *P* value < 0.05. The F-statistics for the genetic instruments are all over the common threshold of 10, indicating that there is no weak instrumental bias (***Online Supplementary Table S2***).

The MR estimates of blood lipids and lipid-related phenotypes on DVT

There were 17 blood lipids and lipid-related traits that showed genetic causal relationships with DVT according to the IVW results (*P* value < 2.64×10^{-4}, ***Online Supplementary Table S3***). The heterogeneity and pleiotropy test found that all *P* values of MR-Egger intercepts and Q statistics were greater than 0.05, while the *P* values for the Q-Q' of 4 phosphatidylcholines (PC aa C34:2, PC ae C36:2, PC ae C38:2, PC aa C36:3) were less than 0.05, indicating the presence of pleiotropy (***Online Supplementary Table S3***). The LOO analysis found that 8 phosphatidylcholines and 1 FA-related trait had main effect SNPs, contributing to the instability of the corresponding MR estimates (***Online Supplementary Figure S2***). Additionally, the MR analysis for sphingomyelin ceramide 16:1 to DVT included only two IVs, allowing

for IVW model analysis exclusively. It was impossible to evaluate heterogeneity and sensitivity, thus subsequent analyses were not included (***Online Supplementary Table S3*).**

After removing 14 unstable exposures, we ultimately identified 3 FA saturation-related traits (Ratio of bis-allylic bonds to double bonds in lipids, Other polyunsaturated fatty acids than 18:2, Ratio of bis-allylic bonds to total fatty acids in lipids) that showed a positive correlation with the risk for DVT (Figure 3). The results of MR-RAPS and weighted median methods for these 3 traits also met the strict threshold of significance (*P* value < 2.64×10^{-4}), and all of them showed a suggestive causality to DVT in weighted mode and MR-Egger methods (*P* value < 0.05). The F-statistics for the genetic instruments are all greater than 10, indicating that there is no weak instrumental bias (***Online Supplementary Table S3***).

The MR estimates of blood lipids and lipid-related phenotypes on PE

The complete results of MR analysis and pleiotropy evaluation for 189 blood lipids and lipid-related traits on PE can be found in ***Online Supplementary Table S4*.** After Bonferroni correction (*P* value < 2.64×10^{-4}), we found that two phosphatidylcholines (PC aa C42:6, PC ae C36:4) and two FA saturation-related traits (Ratio of bis-allylic bonds to double bonds in lipids, Ratio of bis-allylic bonds to total fatty acids in lipids) were positively correlated with PE using IVW, MR-RAPS, and weighted median methods (**Figure 4**). The MR-Egger intercepts, Q statistics and the difference Q-Q' showed that no significant pleiotropy was detected in these MR results (***Online Supplementary Table S4***). The leave-one-out permutation did not identify any IV with major effects in MR estimation (***Online Supplementary Figure S3***). The F-statistics for the genetic instruments are all over the common threshold of 10, indicating that there is no weak instrumental bias (***Online Supplementary Table S4***). These results confirmed the reliability of putative causal effects in our MR analyses.

Discussion

In our study, we employed a two-sample MR approach to investigate the potential causal relationships between 241 blood lipids and lipid-related traits on VTE, DVT and PE. Our findings suggested that higher lipid unsaturation was linked to an increased risk of VTE, DVT and PE. Furthermore, we have revealed a causal relationship of phosphatidylcholines on VTE and PE. MR estimates of medium LDL also demonstrate a protective effect to VTE. The current study provides a foundation to explore the metabolic risk factors of VTE, DVT and PE from the perspective of genetic mechanisms, which is helpful to guide future hypothesis-driven analyses. We have also summarized the biological insights or observational studies related to the causal relationship outcomes in **Table 1** for reference.

Two traits associated with FA saturation (Ratio of bis-allylic bonds to double bonds in lipids, Ratio of bis-allylic bonds to total fatty acids in lipids) showed a significant pathogenic effect on VTE, and another FA saturation-related trait (Other polyunsaturated fatty acids than 18:2) also had an impact on increasing the risk of DVT, indicating that the degree of unsaturation in lipids may be a risk factor for VTE, DVT and PE. The number of double bonds is related to the degree of unsaturation of fatty acids, and the bis-allylic bonds refer to the presence of adjacent double bonds in a molecule. There is little research directly exploring the relationship between lipid unsaturation characteristics and the risk of VTE, but previous studies on the relationship between polyunsaturated fatty acids (PUFA) and VTE seem to support our MR estimates. Maria et al explored the involvement of PUFA biosynthesis in cardiovascular diseases in Europeans and East Asians and found that higher PUFA biosynthesis rates

were associated with a higher risk of VTE.[25] Arachidonic acid (AA) is the major PUFA that undergoes enzymatic oxidation, with cyclooxygenase and lipoxygenase enzymes extracting hydrogen atoms from its bis-allylic carbons to initiate oxidation, generating lipid radicals that then react with molecular oxygen.[26] Higher levels of AA in the serum have been reported for association with a higher risk of VTE.[27,28] The mechanism by which lipid unsaturation affects the risk of VTE may be related to oxidative stress. The presence of bis-allylic methylene between double bonds weakens the carbon-hydrogen bonds, forms carbon-centered radicals and/or hydroperoxides of unsaturated FA, which initiate radical-mediated chain reactions leading to a greater susceptibility of FA to oxidation.[29] The oxidation of certain lipids produces substances with platelet-stimulating properties, such as the oxidation of LDL, which generates lysophosphatidylcholine, some oxidized phosphatidylcholine molecules, and lysophosphatidic acid (LPA). These lipoproteins or lipids activate platelets by stimulating G protein-coupled LPA receptors and the Rho/Rho kinase signaling pathway, resulting in platelet shape change and subsequent aggregation.[30] The more unsaturated fatty acid chains in lipid, the more likely it is to be oxidized to produce reactive substances that promote platelet aggregation. Platelets are essential in hemostasis and are involved in thrombus formation through various mechanisms, including collagen-mediated activation occurring when collagen is exposed beneath the endothelium, adhesion mediated by ultra-large von Willebrand factor (vWF) multimers, and platelet thrombus formation facilitated by neutrophil extracellular traps (NETs).[31] In addition, the formation of certain lipid oxidation products can generate an excess of reactive oxygen species. These free radicals may damage vascular function, increase endothelial permeability, alter responsiveness to vasodilators, and promote the development of focal endothelial cell membrane lesions at very low levels through increased vascular relaxation and platelet aggregation.[32] These events contribute to the progression of VTE by facilitating a series of events that support the formation of venous thrombosis.

In this study, we have identified eight lipid-VTE pairs and nine lipid-DVT pairs containing main SNPs by using leave-one-out analysis. The MR results of these pairs may be driven by the pleiotropic effects of the specific variants rather than the causal effects of the risk factors. We annotated the located genes of these main SNPs in ***Online Supplementary Table S5*** and found that some SNPs are located within specific genes related to lipid unsaturation. For example, rs174546 serves as the influential SNP driving the causal relationship between the trait of double bonds in fatty acids and VTE, while rs174547 drives the causal relationship between traits related to lipid unsaturation (other polyunsaturated fatty acids than 18:2 and CH2 groups to double bonds) and VTE. Both SNPs are located in fatty acid desaturase 1 (*FADS1*) gene. This gene encodes a protein belonging to the fatty acid desaturase gene family, which regulates fatty acid unsaturation by introducing double bonds at specific carbons of the fatty acyl chain.[33,34] Therefore, for exposures related to fatty acid unsaturation, SNPs located in this gene might be used as suitable IVs.

Two phosphatidylcholines (PC aa C42:6, PC ae C36:4) had a positive causal relationship with PE, and PC ae C40:4 showed a negative correlation with VTE. Our results showed that the causality of PC with different carbon chain lengths and double bond numbers on VTE and PE are vary in both positive and negative directions. There is limited direct observational evidence suggesting an association between PC and PE. However, previous metabolomics studies have revealed a correlation between PC and the risk of venous thrombus formation. Sung et al[35] performed metabolomics study using serum and vascular wall extracted from DVT mice, and found that multiple phosphatidylcholines were higher in

DVT mice. The specific mechanism by which phosphatidylcholines increase the risk of PE may be related to their stimulation of platelet aggregation. The PUFA chains of phosphatidylcholines are oxidized to produce highly reactive decomposition products such as malondialdehyde and 4-hydroxynonenal, which potentiate platelet aggregation and thromboxane A2 formation in low concentration ranges.[36,37] The alkyl-phosphatidylcholine and acyl-phosphatidylcholine oxidation products oxidize platelet activating factor (PAF) receptors or induce alterations in human platelet shape, which subsequently stimulate platelet aggregation and thus inducing thrombosis.[38] Moreover, several distinct clinical studies and cohorts have shown that phosphatidylcholine and choline are metabolized by the intestinal microbiota to form the gas trimethylamine (TMA), which is absorbed into the blood and converted to trimethylamine N-oxide (TMAO) by hepatic flavin monooxygenases.[39] TAMO promotes thrombosis in vivo by stimulating Ca^{2+} release from intracellular stores and regulating platelet hyperreactivity and clot formation rate.[40] Whether phosphatidylcholine affects thrombosis through TAMO remains to be considered. It should be noticed that the effects of endogenous phosphatidylcholines may not be identical to dietary intake. Additionally, the underlying mechanisms explaining inhibitory associations between phosphatidylcholine and VTE are far from clear. Previous studies have found that the phosphatidylcholine fraction of various yoghurts, such as PC (18:0/16:0) and PC (18:0/18:1), were inversely correlated with PAF and thrombin inhibition,[41] and the same findings have been found for Salmon phosphatidylcholine.[42] The negative correlation between phosphatidylcholine and VTE requires further experimental and clinical verification.

Our findings also identified negative causality of three phenotypes associated with medium LDL on VTE, including total lipids in medium LDL, concentration of medium LDL particles, and total cholesterol in medium LDL. LDL in plasma is a heterogeneous collection of particles, with differences in size, density, and composition among different subgroups of LDL particles.[43] Subfractions of LDL characterized by particle size, particle number, and lipid composition have different effects on disease. Although the mechanisms underlying the association between medium LDL and VTE are less directly elucidated, numerous studies have shown a significant association between medium LDL and cardiovascular events,[44,45] likely through their effects on endothelial cell function and lipid metabolism influencing thrombosis formation.[46] Previous research has shown that medium-sized and medium-density LDL particles exhibit stronger binding affinity to LDL receptors compared to large, buoyant and small, dense LDL particles.[47,48] LDL particles are highly sensitive to oxidative damage, and oxidized LDL (ox-LDL) is the primary modified form of native LDL.[49] Ox-LDL has been proven to induce changes in platelet shape and aggregation, as well as to transform endothelial cells from an anticoagulant phenotype to a procoagulant phenotype, directly or indirectly promoting coagulation and thrombus formation.[30,37] Therefore, the stronger binding affinity of medium LDL to LDL receptors may help reduce the generation of oxidized LDL, thereby lowering the risk of VTE.

Several limitations should be addressed in current study. Firstly, only 241 blood lipids and related traits were included in our study. More metabolites should be included in future studies, which is important for a more comprehensive understanding of the risk factors and underlying mechanisms of VTE. Secondly, MR studies can help determine whether the observed correlation has a causal relationship based on genetic evidence, which is considered as a causal hypothesis. To confirm the exact causal relationship between specific lipid forms and VTE, more laboratory researches and clinical studies are often needed to reveal potential biological mechanisms. Although our study explored the potential

mechanisms behind the causal relationship between specific lipid forms and VTE, further clinical trials and mechanistic studies are needed for validation.

In conclusion, our study provides MR evidence supporting a causal role of phosphatidylcholine and lipid unsaturation in VTE, DVT, and PE. We hope to get a better understanding of the metabolic mechanisms underlying VTE and predict potential risk factors.

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Table 1. Summary of the literature supporting findings of causality between specific lipids and venous thromboembolism (VTE), deep vein thrombosis (DVT) and pulmonary embolism (PE).

MR results from the current study		Information from previous observational studies	
Exposure	Outcome	Ref.	Conclusion
Lipid unsaturated characters (Ratio of bis-allylic bonds to double bonds in lipids, Ratio of bis-allylic bonds to total fatty acids in lipids)	VTE	Borges et al[25]	Higher rates of polyunsaturated fatty acid biosynthesis rate were associated with a higher risk of VTE.
		Yuan et al[28]	Higher levels of arachidonic acid and stearic acid were associated with a higher chance of VTE.
		Hiki et al[27]	Patients with acute VTE had higher serum levels of arachidonic acid (AA), which accelerates platelet aggregation and inflammation and is processed by the body into various pro-inflammatory and pro-thrombotic metabolites, which contribute to the development of VTE.
Phosphatidylcholines	VTE	Fraser et al[8]	Phosphatidylcholine (PC) was higher in most of the historical patients with VTE.
	DVT	Sung et al[35]	Multiple phosphatidylcholines were higher in DVT mice.
	DVT	Gu et al[50]	The level of phosphatidylcholine 22:6/20:2 (PC 22:6/20:2) was significantly reduced in the DVT rat model group.
Phenotypes associated with medium low density lipoprotein (LDL)	VTE	Pichler et al[44]	Among the LDL particle subclasses, medium LDL particles showed the strongest association with cardiovascular events.
		Musunuru et al[45]	Medium LDL was most highly associated with risk for cardiovascular disease.
		Mora et al[46]	Different particle subclasses and particle sizes of LDL affect thrombus formation by affecting endothelial cell function and lipid metabolism.

Highlight

- Mendelian randomization estimation revealed the traits associated with fatty acid unsaturation had positively causality on VTE, DVT and PE, suggesting that fatty acid unsaturation may be a risk factor of VTE.
- Several phosphatidylcholines (PC ae C40:4, PC aa C42:6, PC ae C36:4) with FA chains containing different numbers of carbon atoms and double bonds had different effects on the risk of VTE and PE.
- Several traits associated with medium LDL (Total lipids in medium LDL, Concentration of medium LDL particles, Total cholesterol in medium LDL) had a protective effect on VTE

• Figure Legends

Figure 1. Study design. GWAS: genome-wide association study; VTE: venous thromboembolism; DVT: deep vein thrombosis; PE: pulmonary embolism; IV: instrumental variable; MR: Mendelian randomization; IVW: inverse-variance weighted; MR-RAPS: Mendelian randomization robust adjusted profile score.

Figure 2. Causal effects of 1 phosphatidylcholine, 2 traits related to double bond composition and 3 traits related medium low density lipoprotein (LDL) on venous thromboembolism (VTE). Summary MR estimates derived from the IVW, MR-RAPS, weighted median, weighted mode, and MR-Egger methods. The error bars represent 95% confidence intervals. MR: Mendelian randomization; IVW: inverse-variance weighted; MR-RAPS: Mendelian randomization robust adjusted profile score.

Figure 3. Causal effects of 3 traits related to fatty acid unsaturation on deep vein thrombosis (DVT). Summary MR estimates derived from the IVW, MR-RAPS, weighted median, weighted mode, and MR-Egger methods. The error bars represent 95% confidence intervals. MR: Mendelian randomization; IVW: inverse-variance weighted; MR-RAPS: Mendelian randomization robust adjusted profile score.

Figure 4. Causal effects of 2 phosphatidylcholines and 2 traits related to double bond composition on pulmonary embolism (PE). Summary MR estimates derived from the IVW, MR-RAPS, weighted median, weighted mode, and MR-Egger methods. The error bars represent 95% confidence intervals. MR: Mendelian randomization; IVW: inverse-variance weighted; MR-RAPS: Mendelian randomization robust adjusted profile score.

Figure 1

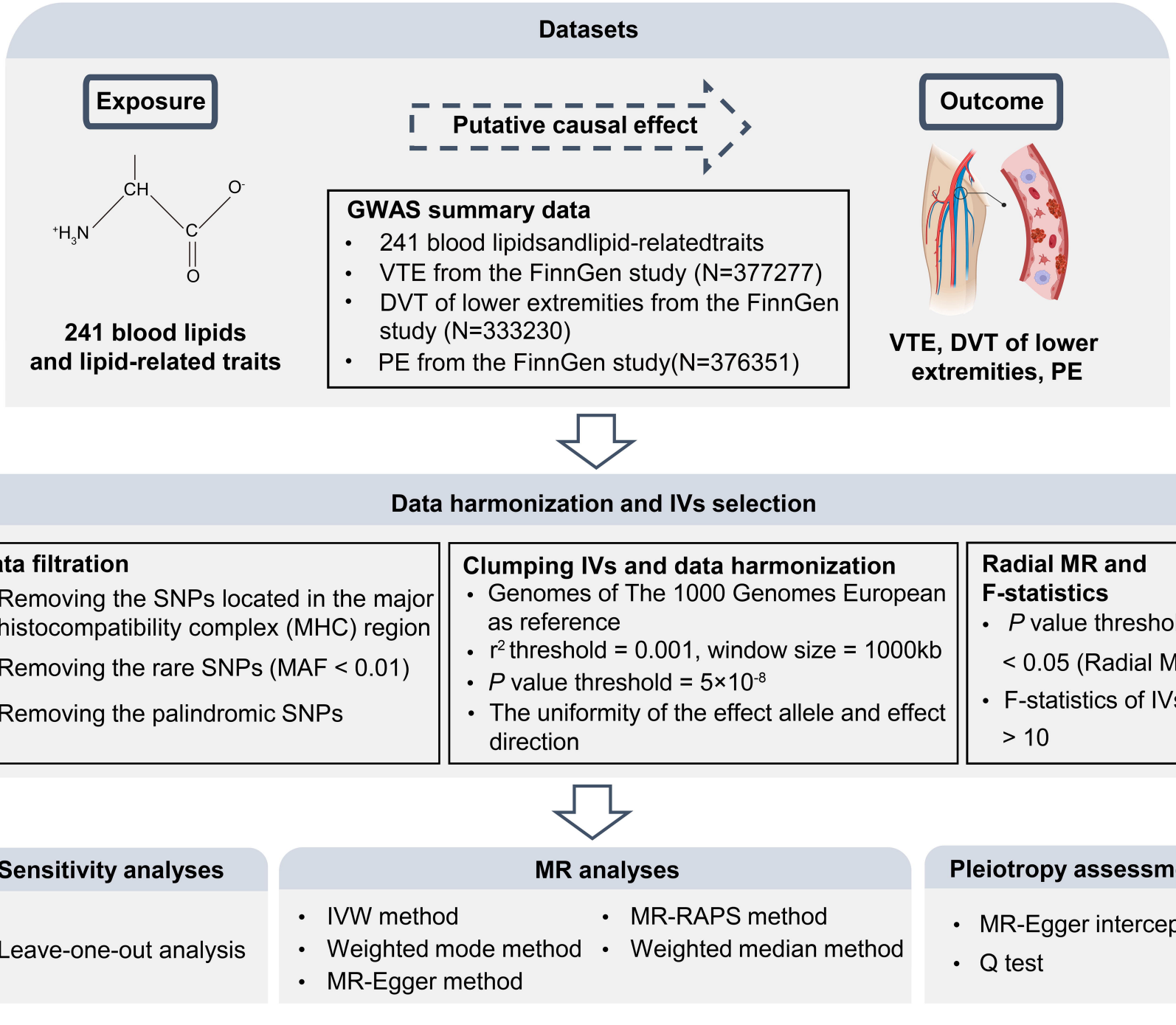

Datasets
Exposure
Putative causal effect
Outcome
241 blood lipids and lipid-related traits
GWAS summary data
241 blood lipidsandlipid-relatedtraits
VTE from the FinnGen study (N=377277)
DVT of lower extremities from the FinnGen study (N=333230)
PE from the FinnGen study(N=376351)
VTE, DVT of lower extremities, PE
Data harmonization and IVs selection
Data filtration
Removing the SNPs located in the major histocompatibility complex (MHC) region
Removing the rare SNPs (MAF < 0.01)
Removing the palindromic SNPs
Clumping IVs and data harmonization
Genomes of The 1000 Genomes European as reference
r2 threshold = 0.001, window size = 1000kb
P value threshold = 5×10-8
The uniformity of the effect allele and effect direction
Radial MR and F-statistics
P value threshold < 0.05 (Radial MR)
F-statistics of IVs > 10
Sensitivity analyses
Leave-one-out analysis
MR analyses
IVW method
Weighted mode method
MR-Egger method
MR-RAPS method
Weighted median method
Pleiotropy assessment
MR-Egger intercept
Q test

Figure 2

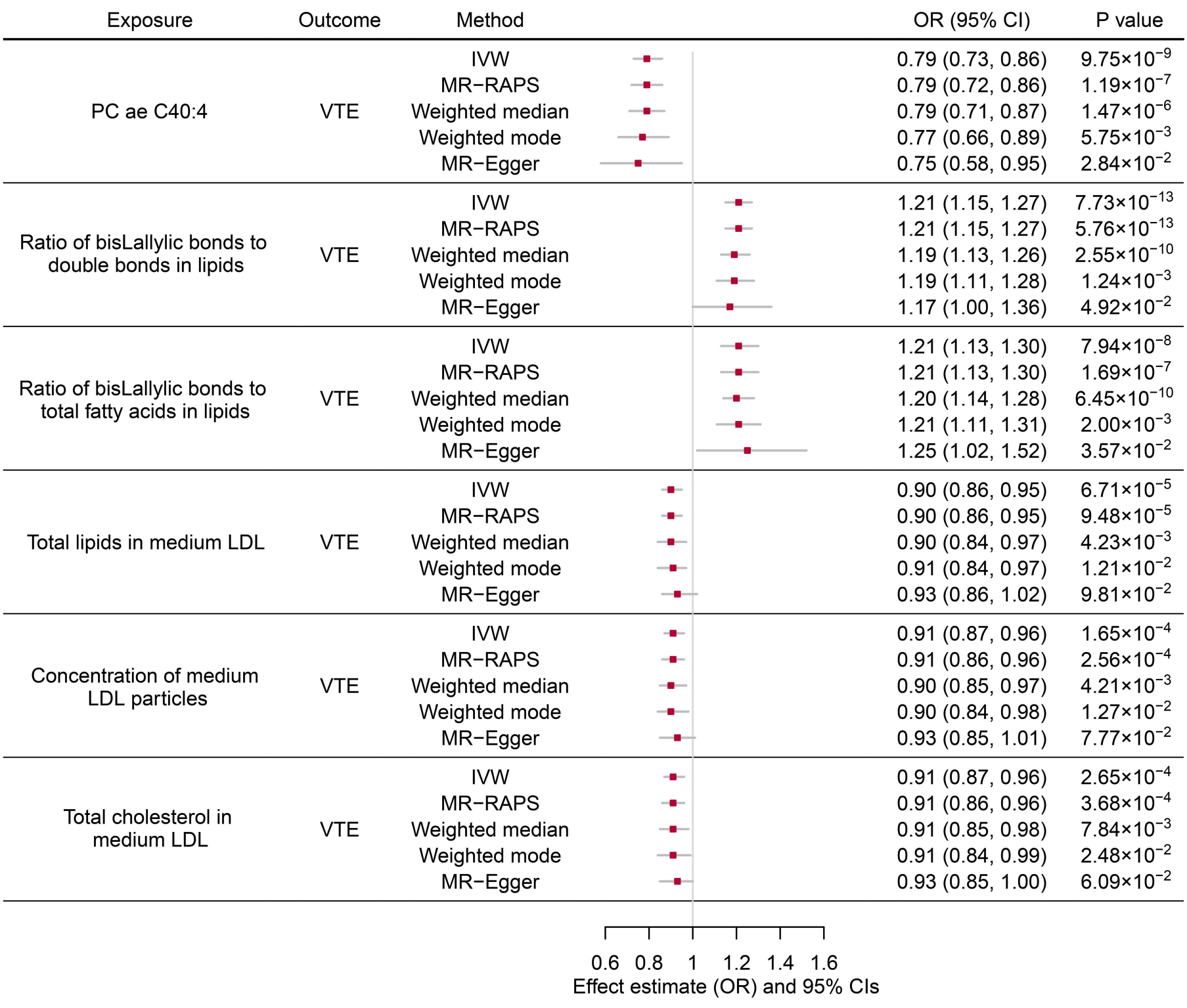

Exposure
Outcome
Method
OR (95% CI)
P value
PC ae C40:4
VTE
IVW
0.79 (0.73, 0.86)
9.75×10−9
MR−RAPS
0.79 (0.72, 0.86)
1.19×10−7
Weighted median
0.79 (0.71, 0.87)
1.47×10−6
Weighted mode
0.77 (0.66, 0.89)
5.75×10−3
MR−Egger
0.75 (0.58, 0.95)
2.84×10−2
Ratio of bisLallylic bonds to double bonds in lipids
VTE
IVW
1.21 (1.15, 1.27)
7.73×10−13
MR−RAPS
1.21 (1.15, 1.27)
5.76×10−13
Weighted median
1.19 (1.13, 1.26)
2.55×10−10
Weighted mode
1.19 (1.11, 1.28)
1.24×10−3
MR−Egger
1.17 (1.00, 1.36)
4.92×10−2
Ratio of bisLallylic bonds to total fatty acids in lipids
VTE
IVW
1.21 (1.13, 1.30)
7.94×10−8
MR−RAPS
1.21 (1.13, 1.30)
1.69×10−7
Weighted median
1.20 (1.14, 1.28)
6.45×10−10
Weighted mode
1.21 (1.11, 1.31)
2.00×10−3
MR−Egger
1.25 (1.02, 1.52)
3.57×10−2
Total lipids in medium LDL
VTE
IVW
0.90 (0.86, 0.95)
6.71×10−5
MR−RAPS
0.90 (0.86, 0.95)
9.48×10−5
Weighted median
0.90 (0.84, 0.97)
4.23×10−3
Weighted mode
0.91 (0.84, 0.97)
1.21×10−2
MR−Egger
0.93 (0.86, 1.02)
9.81×10−2
Concentration of medium LDL particles
VTE
IVW
0.91 (0.87, 0.96)
1.65×10−4
MR−RAPS
0.91 (0.86, 0.96)
2.56×10−4
Weighted median
0.90 (0.85, 0.97)
4.21×10−3
Weighted mode
0.90 (0.84, 0.98)
1.27×10−2
MR−Egger
0.93 (0.85, 1.01)
7.77×10−2
Total cholesterol in medium LDL
VTE
IVW
0.91 (0.87, 0.96)
2.65×10−4
MR−RAPS
0.91 (0.86, 0.96)
3.68×10−4
Weighted median
0.91 (0.85, 0.98)
7.84×10−3
Weighted mode
0.91 (0.84, 0.99)
2.48×10−2
MR−Egger
0.93 (0.85, 1.00)
6.09×10−2
0.6
0.8
1
1.2
1.4
1.6
Effect estimate (OR) and 95% CIs

Figure 3

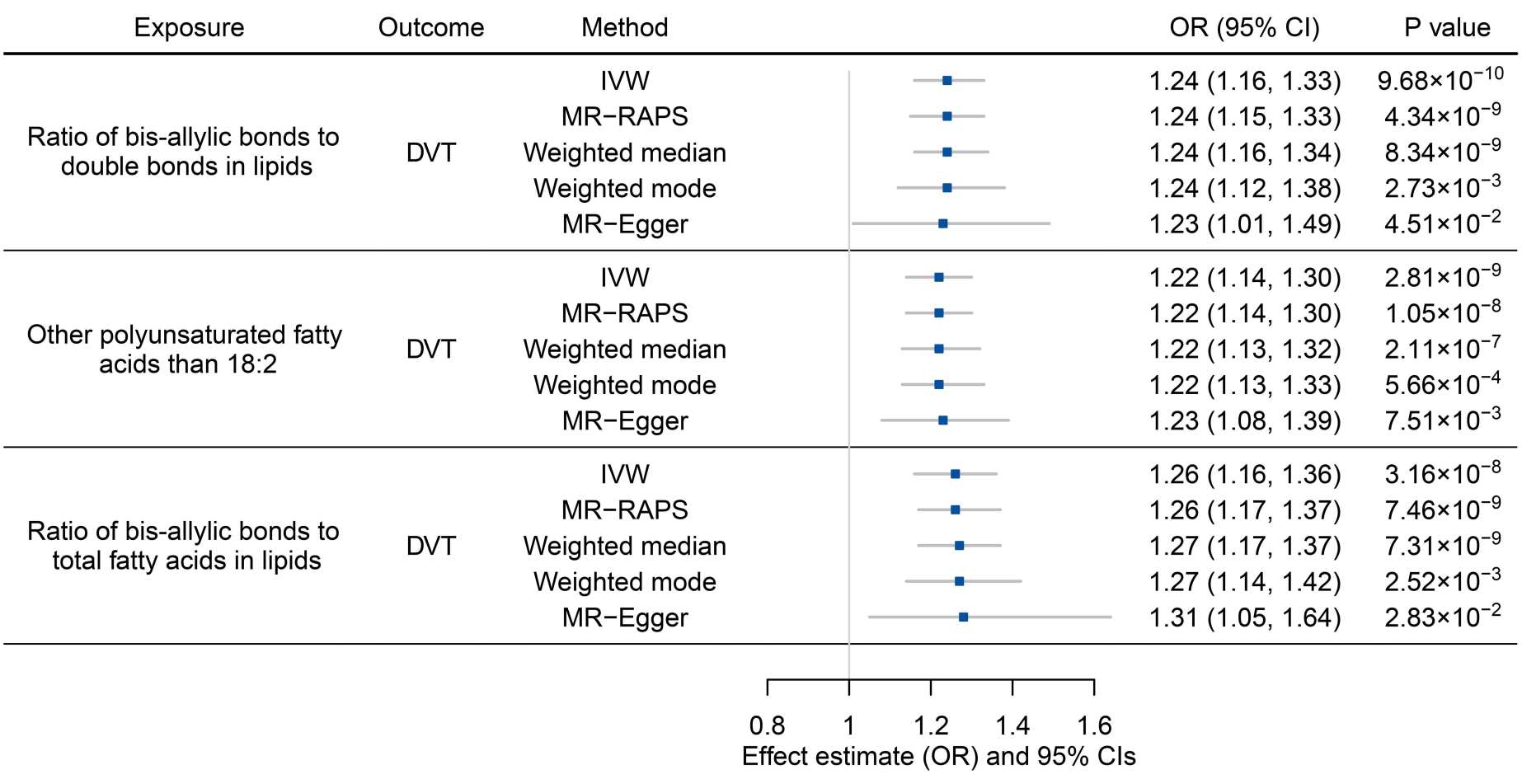
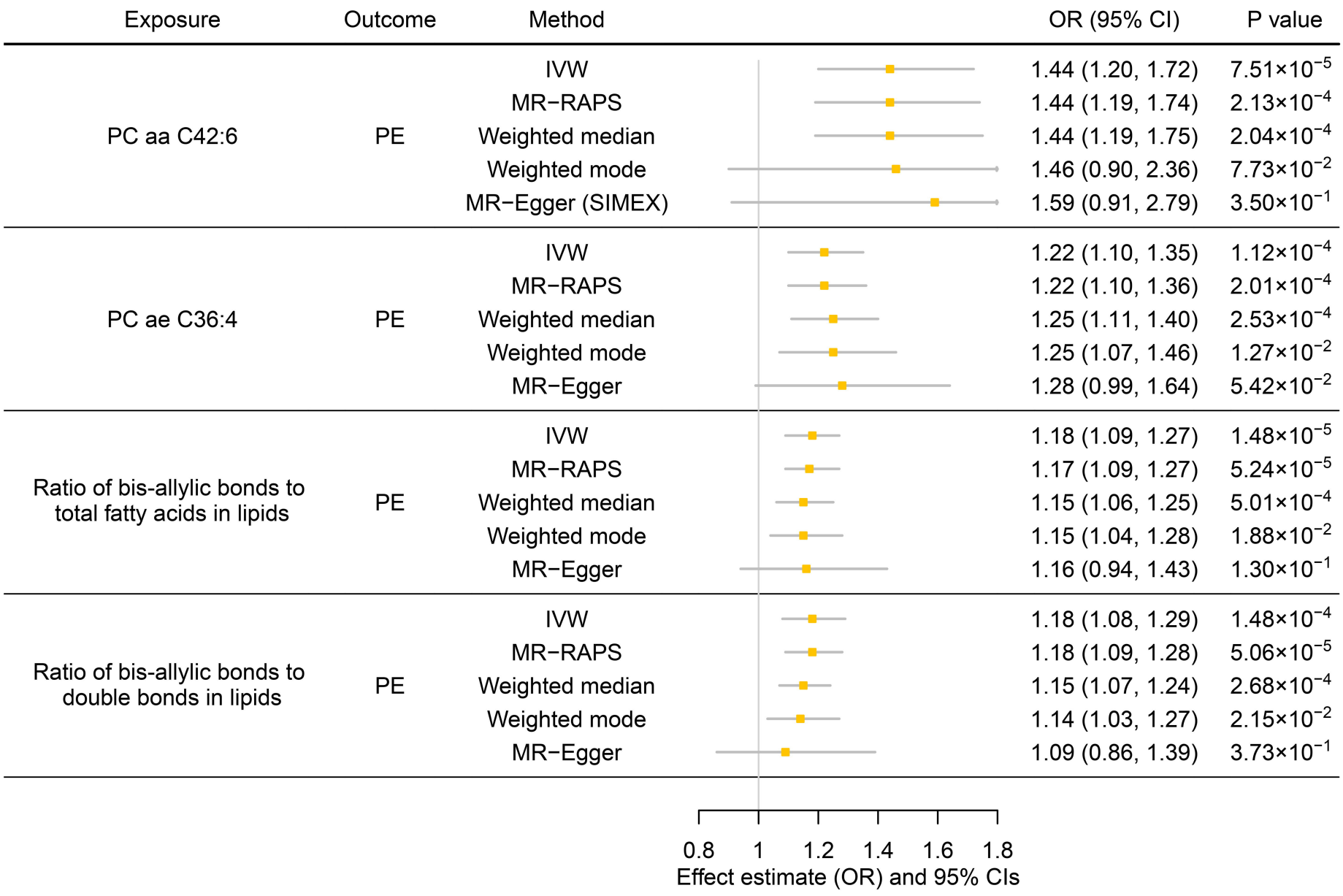

Exposure
Outcome
Method
OR (95% CI)
P value
Ratio of bis-allylic bonds to double bonds in lipids
DVT
IVW
1.24 (1.16, 1.33)
9.68×10−10
MR−RAPS
1.24 (1.15, 1.33)
4.34×10−9
Weighted median
1.24 (1.16, 1.34)
8.34×10−9
Weighted mode
1.24 (1.12, 1.38)
2.73×10−3
MR−Egger
1.23 (1.01, 1.49)
4.51×10−2
Other polyunsaturated fatty acids than 18:2
DVT
IVW
1.22 (1.14, 1.30)
2.81×10−9
MR−RAPS
1.22 (1.14, 1.30)
1.05×10−8
Weighted median
1.22 (1.13, 1.32)
2.11×10−7
Weighted mode
1.22 (1.13, 1.33)
5.66×10−4
MR−Egger
1.23 (1.08, 1.39)
7.51×10−3
Ratio of bis-allylic bonds to total fatty acids in lipids
DVT
IVW
1.26 (1.16, 1.36)
3.16×10−8
MR−RAPS
1.26 (1.17, 1.37)
7.46×10−9
Weighted median
1.27 (1.17, 1.37)
7.31×10−9
Weighted mode
1.27 (1.14, 1.42)
2.52×10−3
MR−Egger
1.31 (1.05, 1.64)
2.83×10−2
0.8
1
1.2
1.4
1.6
Effect estimate (OR) and 95% CIs

Figure 4


Exposure
Outcome
Method
OR (95% CI)
P value
PC aa C42:6
PE
IVW
1.44 (1.20, 1.72)
7.51×10−5
MR−RAPS
1.44 (1.19, 1.74)
2.13×10−4
Weighted median
1.44 (1.19, 1.75)
2.04×10−4
Weighted mode
1.46 (0.90, 2.36)
7.73×10−2
MR−Egger (SIMEX)
1.59 (0.91, 2.79)
3.50×10−1
PC ae C36:4
PE
IVW
1.22 (1.10, 1.35)
1.12×10−4
MR−RAPS
1.22 (1.10, 1.36)
2.01×10−4
Weighted median
1.25 (1.11, 1.40)
2.53×10−4
Weighted mode
1.25 (1.07, 1.46)
1.27×10−2
MR−Egger
1.28 (0.99, 1.64)
5.42×10−2
Ratio of bis-allylic bonds to total fatty acids in lipids
PE
IVW
1.18 (1.09, 1.27)
1.48×10−5
MR−RAPS
1.17 (1.09, 1.27)
5.24×10−5
Weighted median
1.15 (1.06, 1.25)
5.01×10−4
Weighted mode
1.15 (1.04, 1.28)
1.88×10−2
MR−Egger
1.16 (0.94, 1.43)
1.30×10−1
Ratio of bis-allylic bonds to double bonds in lipids
PE
IVW
1.18 (1.08, 1.29)
1.48×10−4
MR−RAPS
1.18 (1.09, 1.28)
5.06×10−5
Weighted median
1.15 (1.07, 1.24)
2.68×10−4
Weighted mode
1.14 (1.03, 1.27)
2.15×10−2
MR−Egger
1.09 (0.86, 1.39)
3.73×10−1
0.8
1
1.2
1.4
1.6
1.8
Effect estimate (OR) and 95% CIs

Supplementary Material

Two-sample Mendelian randomization analysis reveals causal relationships between blood lipids and venous thromboembolism

Contents

Supplementary Methods

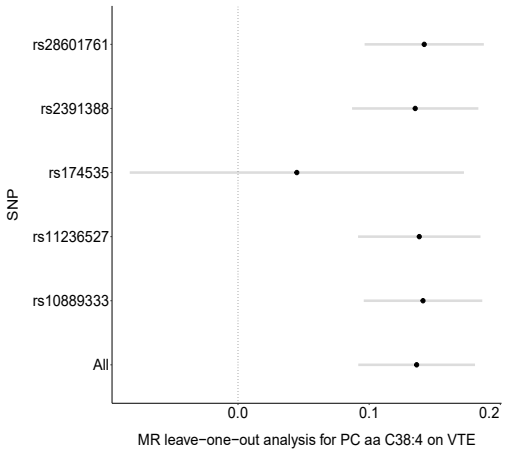
Genetic Instruments Selection and Data Harmonization

Using three-step approaches, we obtained the effective instrumental variables (IVs) of each exposure. Genetic IVs must conform to three hypotheses: (1) have a strong robust correlation with exposure; (2) were independent of confounding associated with exposures and outcomes; (3) only affect the outcome through exposures, but not through other ways[3]. According to these hypotheses, we first selected independent SNPs by clump algorithm module of plink1.9 software[4]. The 1000 Genomes European data was used as reference the panel for linkage disequilibrium (LD) estimation (r^2 threshold = 0.001, window size = 1000kb, *P* value threshold = 5×10^{-8}). Next, we performed a heterogeneity test using the RadialMR package[5] which identified outlier pleiotropic SNPs via modified Q statistics. The threshold for outlier definition is *P* value < 0.05. Finally, we used F-statistics to evaluate the IVs strength for each exposure, while an F-statistic < 10 was considered to be weak intensity[6]. After IVs selection, we harmonized the effect alleles and adjusted *β* values in the outcome data to make it consistent with the exposure data[1].

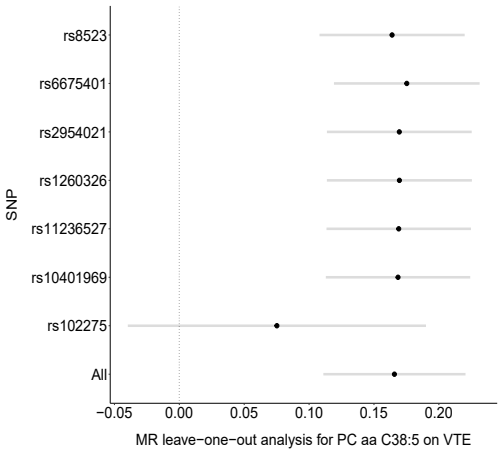
MR Analyses

The IVW method with multiplicative random effects model can be applied to the summary data estimates in the presence of observed heterogeneity[7], which was deemed as the main MR method in our study. The MR-RAPS method is robust to both systematic and idiosyncratic pleiotropy, especially for MR estimation with many weak instruments[8]. It is recommended in cases where exposure and outcomes are both complex traits. MR-Egger method allows all genetic variants to be pleiotropic but requires to be satisfied with the Instrument Strength Independent of Direct Effect (InSIDE) assumption. It assumes that the pleiotropic effect is the same in all variables. This means that pleiotropy leads to bias, but not to additional heterogeneity[7]. The enhancement of the pleiotropy robustness of the MR-Egger method leads to the violation of no measurement error (NOME) in the SNP exposure effects assumption, which can be evaluated by the regression dilution I^2 (GX)[9]. When I^2 (GX) is close to 1, the attenuation due to NOME violation will be negligible. If I^2 (GX) < 0.9, the Simulation Extrapolation (SIMEX) method should be employed to correct this regression dilution bias. Since invalid instrumental variables do not directly affect the median estimate, the weighted median method is able to accurately calculate causal association effects when less than 50% of the genetic variation violates the MR hypothesis[10]. For the weighted mode method, the NOME assumption is not necessary. It relaxed the IV assumption, showing less bias and a lower type I error rate[11].

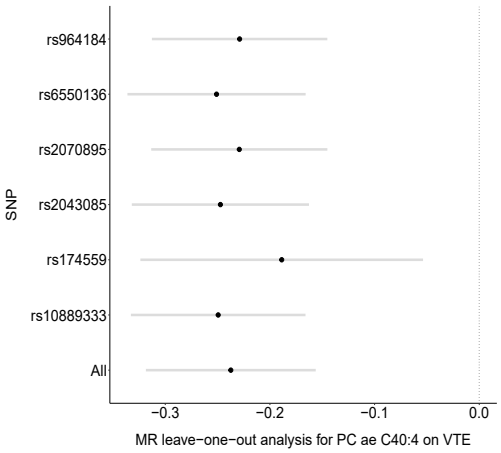
Supplementary Figures



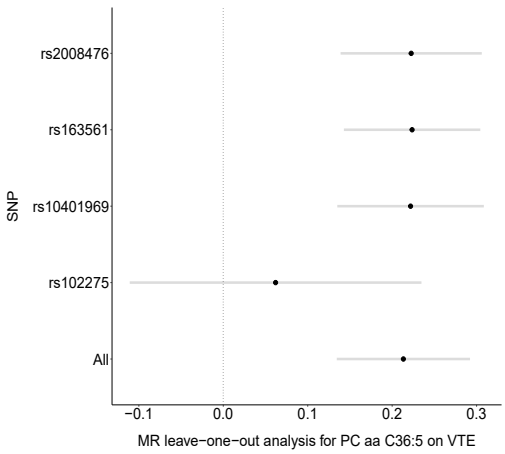
MR leave−one−out analysis for PC aa C38:4 on VTE



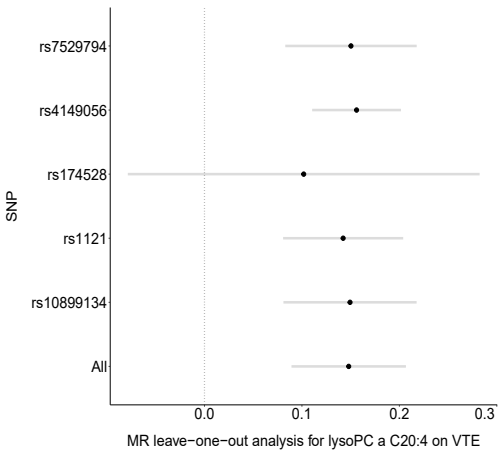
MR leave−one−out analysis for PC aa C38:5 on VTE



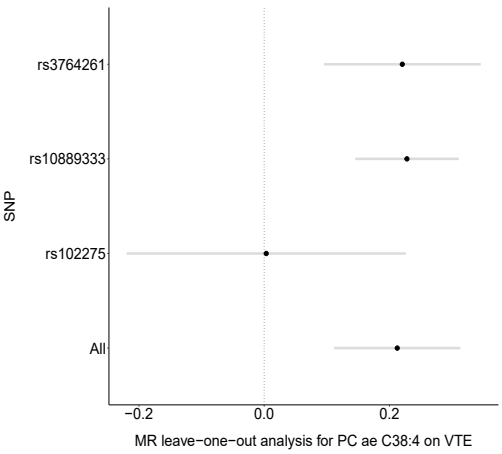
MR leave−one−out analysis for PC ae C40:4 on VTE



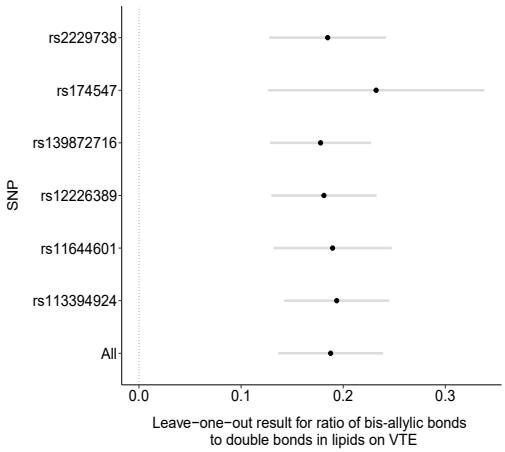
MR leave−one−out analysis for PC aa C36:5 on VTE



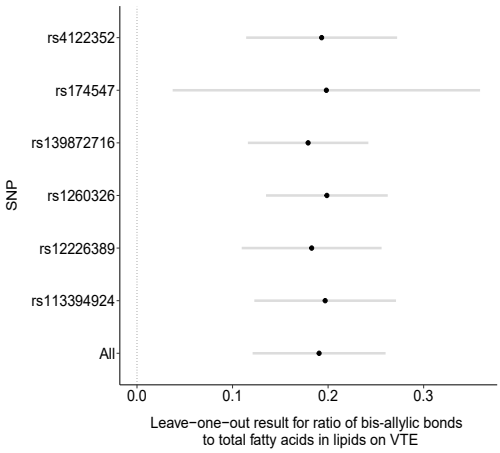
MR leave−one−out analysis for lysoPC a C20:4 on VTE



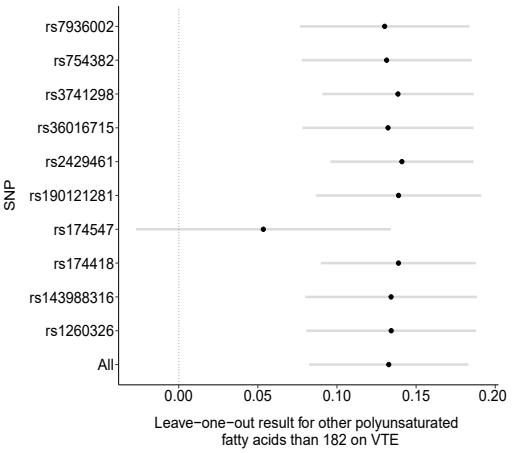
MR leave−one−out analysis for PC ae C38:4 on VTE



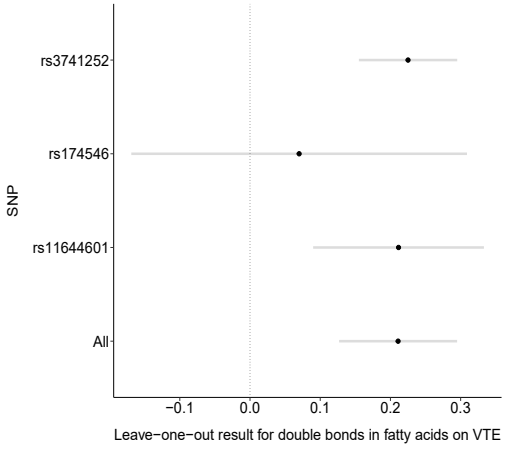
Leave−one−out result for ratio of bis−allylic bonds
to double bonds in lipids on VTE



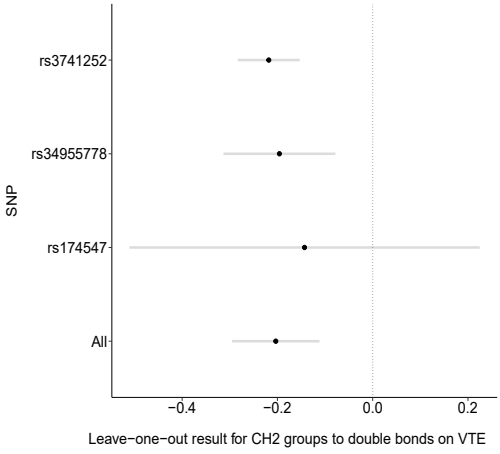
Leave−one−out result for ratio of bis−allylic bonds
to total fatty acids in lipids on VTE



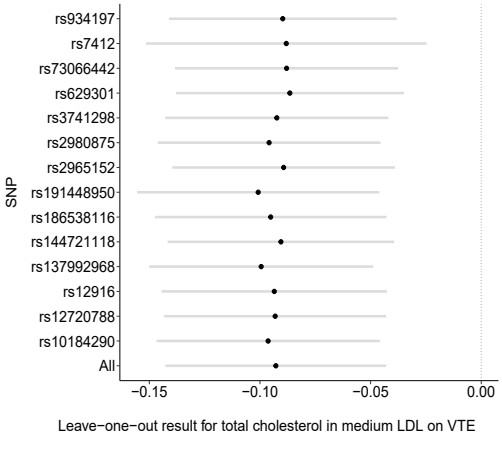
Leave−one−out result for other polyunsaturated
fatty acids than 182 on VTE



Leave−one−out result for double bonds in fatty acids on VTE



Leave−one−out result for CH2 groups to double bonds on VTE



Leave−one−out result for total cholesterol in medium LDL on VTE

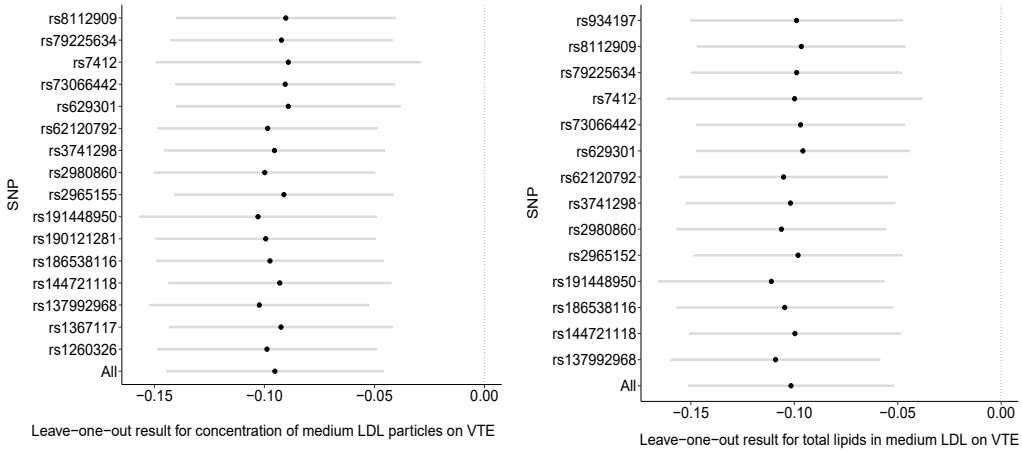


Figure S1. The plot of leave-one-out analysis for 14 significant lipids and lipid-related traits on VTE.

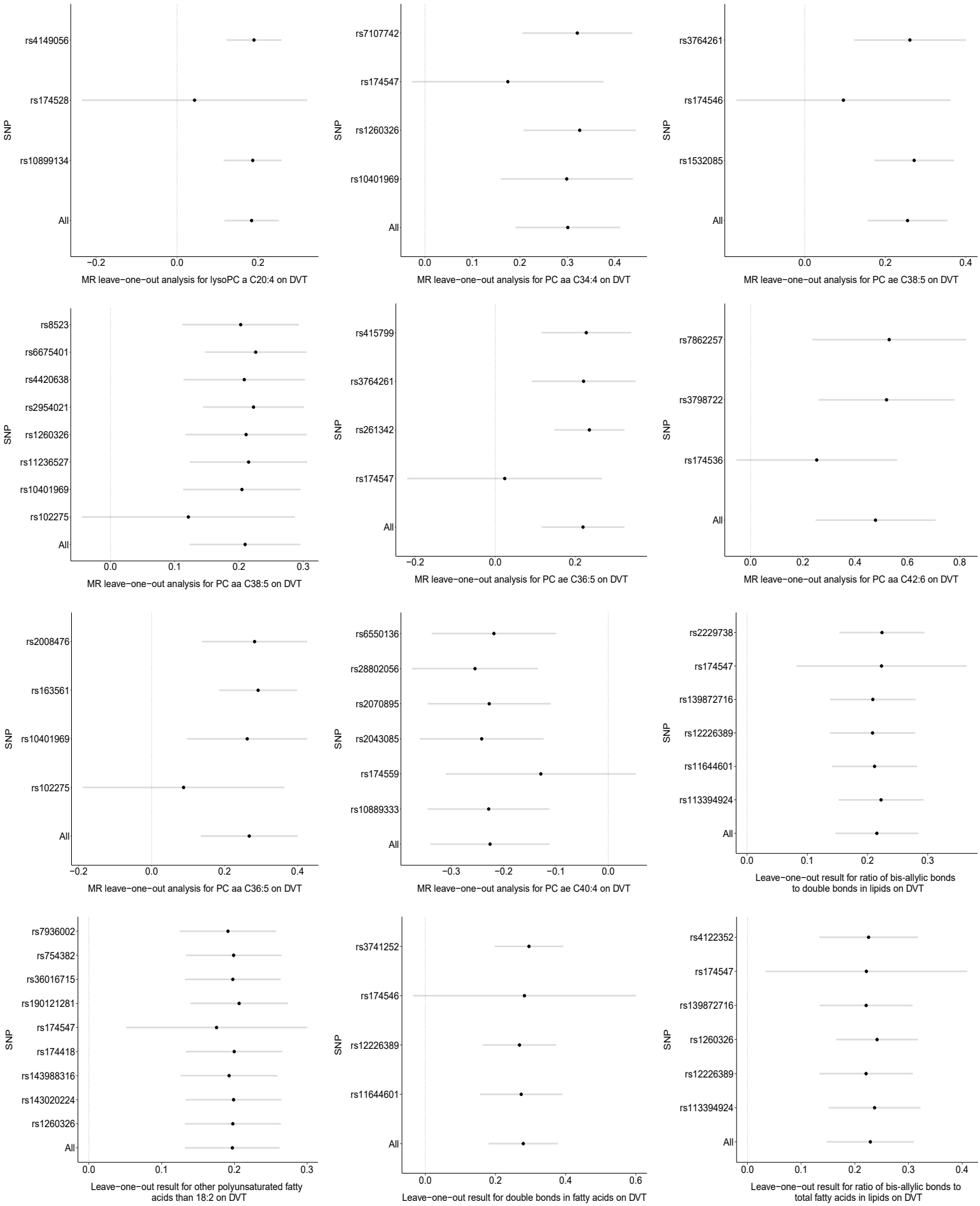


Figure S2. The plot of leave-one-out analysis for 12 significant lipids and lipid-related traits on DVT.

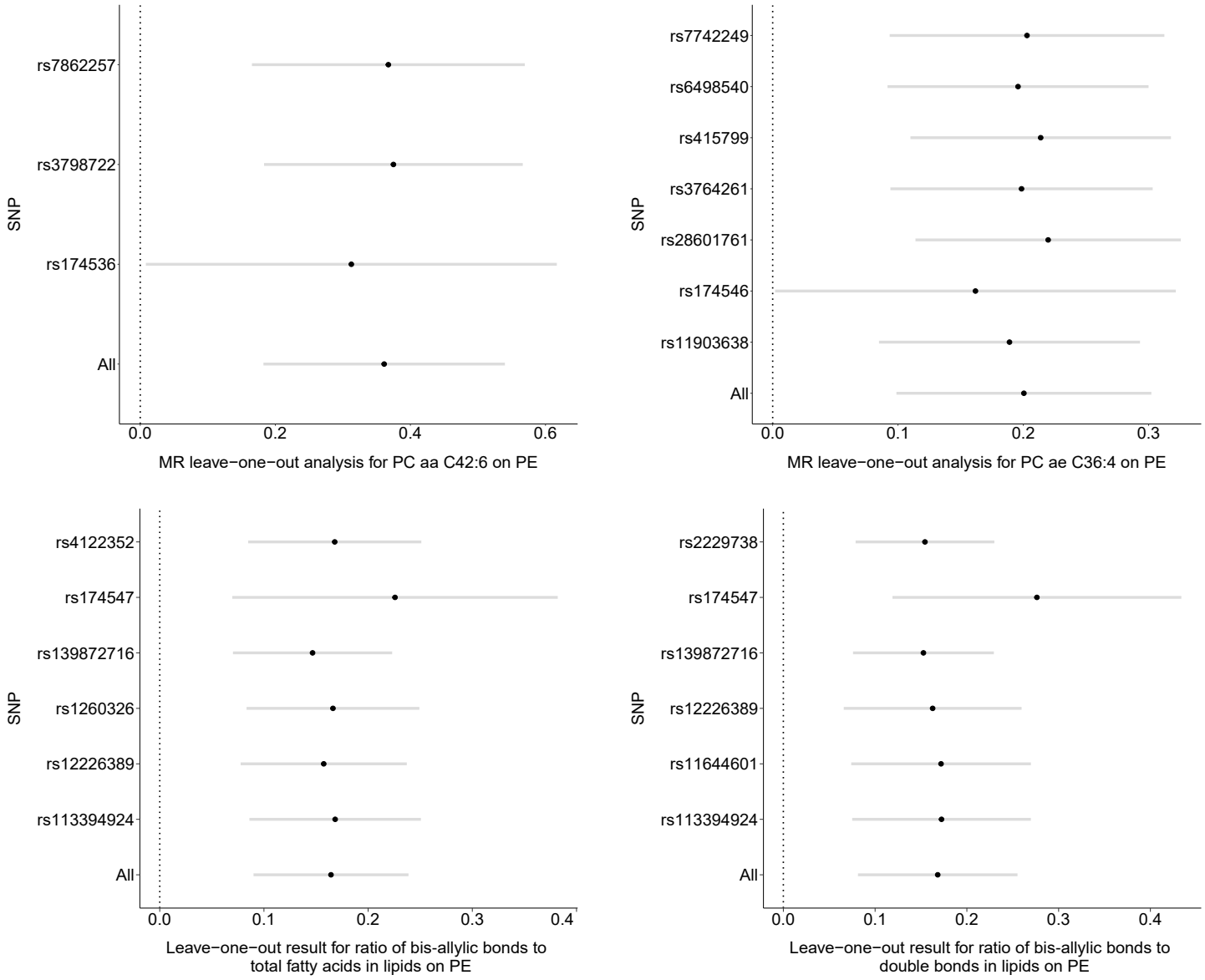


Figure S3. The plot of leave-one-out analysis for 4 significant lipids and lipid-related traits on PE.

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